DALTON (J. C)

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THE present condition of our knowledge on the glycogenic function is as follows :—

It is universally known that the liver in healthy animals, when examined within a few minutes after death, contains an appreciable amount of glucose; that this glucose increases in quantity in the liver tissue after the circulation has ceased; that it will even reappear in the liver, separated from the body, after having been entirely washed out by a continued watery injection of the hepatic vessels; and that it is produced by a catalytic transformation of the amyloid substance, or glycogene, under the influence of an animal ferment. All these facts, due to the remarkable discoveries of Bernard, have been abundantly confirmed by other experimenters, and are established in a manner which leaves no room for question.

It is doubted, however, at the present day, whether glucose really exists in the liver during life, and consequently whether there is any such thing as the glycogenic function, properly speaking. This doubt was first raised in 1858 by Pavy,* who asserted that the glucose found in the liver after death was a substance exclusively of post-mortem production; that the liv-

* Proceedings of the Royal Society of London, 1858. IX., p. 300.

ing organ contained only the amyloid substance, or "hepatine," as he called it, which, however, was transformed into glucose after death with extraordinary rapidity; but that the non-existence of sugar, as a physiological ingredient, could be proved by injecting the liver immediately after death with a solution of potash, or by instantly placing the portion cut off in a freezing mixture of ice and salt, either of which processes would arrest the catalytic transformation of the glycogene. This conclusion was entirely unwarranted by the experiments which Pavy reported, since in no single instance was glucose altogether absent from the liver tissue in healthy animals treated as above, or from the blood of the right ventricle, removed by catheterization during life. It was simply present in very minute quantity, as compared with that found in the organ after a short time had elapsed.

The opinion of Pavy was controverted by Harley in 1860,* who operated by killing the animals by section of the medulla oblongata, immediately placing a portion of the liver in the freezing mixture, and afterward slicing it directly into boiling acidulated water. He reported four of these experiments, in all of which the liver tissue, so treated, gave distinct evidence of sugar. In one of them, the time which elapsed from the death of the animal to the immersion of the liver tissue in the freezing mixture is given as "less than twenty seconds."

Other observers, however, adopted Pavy's view. Meissner,† in 1862, and Ritter,‡ in 1865, repeated the experiments in a slightly modified form, by suddenly slicing out a portion of the liver from the living rabbit, cutting it into small pieces, and immediately dropping it into boiling water. They found that the extract, prepared in this way, gave no reaction when examined by Trommer's test,—and conclude accordingly that the liver of the healthy animal, during life, contains no trace of glucose.

Schiff,§ in 1866, arrived at the same result in dogs, cats, rabbits, and guinea-pigs, by taking out a portion of liver at

* Proceedings of the Royal Society of London. X., p. 289.
† Zeitschrift für rationelle Medicin. XIX., pp. 310, 311, 312.
‡ Zeitschrift für rationelle Medicin. XXIV., p. 65.
§ Journal de l'Anatomie et de la Physiologie. 3me année, No. 4, p. 354.

the instant of death, and cutting it up at once into boiling water.

On the other hand, Eulenberg,* in 1868, on repeating Ritter's experiments, found in every case traces of sugar, provided the copper test were thoroughly applied; and he concludes that the extract of the liver, prepared by the boiling water process, will always show evidence of glucose, if the test be employed with due care. He thinks, however, that the sugar so obtained may have been produced in the few instants of time required for cutting up the organ into small pieces,—and he accordingly adopted the plan of grinding the liver substance in a mortar with alcohol and pounded glass. The extracts which he obtained in this way, in six cases, were entirely destitute of sugar, when examined by the copper test.

Professor Flint, jr.,† in 1868, experimented upon dogs by cutting out a portion of the liver during life, slicing it into boiling water, and examining the extract by Trommer's and Fehling's tests. In two instances, where the time employed in the operation was respectively 28 seconds and 22 seconds, there was no marked or certain evidence of sugar. In one instance, where the time employed was only 10 seconds, the liver extract presented no trace of sugar whatever. The blood of the hepatic veins, however, obtained by ligature of the vena cava inferior within a minute after the first operation, showed a well-marked reaction with the copper test. Professor Flint concludes that the glycogenic matter is really converted into sugar by the liver during life, but is carried away from the organ, as fast as it is formed, by the current of the circulating blood.

Finally, Professor Lusk,‡ in 1870, operated upon five dogs, in order to determine the difference, if any, in the quantity of sugar contained in the blood of the right ventricle, removed by catheterization during life, and that of the jugular vein. He found in every instance, contrary to the results of Pavy, McDonnell, Meissner, and Ritter, that the sugar contained in the hepatic blood preponderated very considerably over that in the general circulation,—and that the quantity of glucose in the blood

* Journal für praktische Chemie. CIII., p. 108.

† New York Medical Journal. January, 1869.

‡ New York Medical Journal. July, 1870.

of the right side of the heart was from two to four times greater than that found under the same circumstances in the jugular vein.

Two years ago, I was desirous of ascertaining the exact time within which glucose would fail to appear in the liver extract examined by the ordinary method. For this purpose I experimented upon dogs by cutting out a portion of the liver in the same manner as Professor Flint had done, slicing it into boiling water, and making an extract of the coagulated liver tissue by rubbing it to a pulp in a mortar, and treating different portions by boiling with pure water, boiling with an excess of sulphate of soda, and lixiviating with cold water through finely powdered animal charcoal. In one instance the liver substance, immediately on being removed from the body, was crushed between two slabs of ground glass, rubbed to a pasty mass with animal charcoal, and then lixiviated with cold water. The result was, that when the preliminary operations were completed in 17 seconds and 22 seconds, the final extract of the liver tissue gave no reduction by the copper test; but at the end of 50 seconds it gave rather slowly a distinct though not abundant indication of sugar. In one instance, different portions of the same liver were treated by boiling water, and afterward with animal charcoal, at the end of 17 seconds, and at the end of one, two, three, four, five, and seven minutes successively. In the first instance (17 seconds), there was no indication of sugar by Trommer's test; in the second (one minute), the sugar reaction was delicate but distinct. In the remaining five specimens the reaction, as appreciated by the eye, was constantly more and more marked. The appearance of the different test tubes, after the completion of the experiment, was very striking. In the first, representing the liver extract at the end of 17 seconds, the liquid remained perfectly blue and transparent; the remainder all showed a yellow or reddish color from the reduction of the copper, and varied only in the intensity of the hue and the quantity of deposit, which increased exactly in proportion to the time which had elapsed before the end of the operation.

According to these results, therefore, 50 seconds was the shortest time within which the liver tissue, removed from the living animal, could be found to give indication of the presence of sugar.

These experiments, however, were not fully satisfactory to me, for several reasons. In the first place, when a substance like glucose invariably appears in an animal tissue after death with such rapidity that the interval is to be counted by seconds, it naturally suggests the propriety of extreme caution in adopting the conclusion that it was not there before,—at least in minute quantity. Especially as nearly all observers are agreed that slight disturbances of the circulation or respiration, the struggles of the animal immediately before the operation, or the compressing effect of ligatures, will cause the appearance of glucose in the liver tissue at the instant of its removal, the necessity of such caution becomes very evident. Schiff states * that in various animals, by simply compressing the abdominal aorta for ten minutes, or tying the principal blood-vessels of one limb, he has produced a condition of diabetes ; and, on killing the animal, has found sugar present in the liver, though examined with all the requisite precautions. Even the use of ether is interdicted in experiments of this nature, owing to its liability to bring on a saccharine condition of the liver during life. According to the original observations of Bernard, the glucose produced in the liver was supposed to be constantly carried away by the blood of the hepatic veins, to be replaced by a fresh supply of new formation ; so that a comparatively large amount of sugar might be supplied by the liver in twenty-four hours, and yet only a small quantity be present in the organ at any one time. We all know that, in point of fact, the amount of sugar in the liver tissue increases after stoppage of the circulation, just as urea accumulates in the blood after removal of the kidneys, or carbonic acid in the lungs after the stoppage of respiration. The question is, whether this increase of sugar is simply the accumulation of a substance already existing in small quantity, or a matter entirely of post-mortem production.

It must be remembered, furthermore, that the chemical tests for glucose, as well as for other substances, have their limits in point of delicacy ; and it is possible that they may fail to detect its presence, in some instances, simply on account of its minute quantity. There was a time when it was impossible to detect

* Journal de l'Anatomie et de la Physiologie. 3me année, No. 4, p. 365.

the presence of urea in healthy blood ; and it was only after the requisite improvement in our chemical manipulations that this substance could be distinguished as a normal ingredient of the circulation. This consideration is of some importance in the present connection, because the quantity of liver tissue examined in the above experiments is of itself necessarily small. I have found it difficult to cut up in sufficiently thin pieces, and immerse in the boiling water within the requisite time, more than about 140 grains of the liver tissue. If a much larger quantity than this be used, it requires more time for completing the operation, and gives rise to the presumption that the sugar afterward found may have been produced by fermentation during the interval which has elapsed.

For these reasons I was anxious, in the first place, to determine the exact limits of sensibility of the various tests for sugar, and the best manner of employing them ; and secondly, to contrive some plan by which a larger quantity of liver tissue might be used for experiment, without increasing the time consumed in the operation.

The most convenient and generally useful of all the means for detecting sugar is that known as Trommer's test. The imperfection in this test, however, is that it is indefinite in regard to quantity. The only rule commonly recognized for its application is to add to the suspected liquid, first, a solution of sulphate of copper, and then a solution of potassa, in sufficient quantity to give to the mixture a clear blue color and a distinctly alkaline reaction. On boiling, if glucose be present, the blue color changes to a light turbid yellow, or an opaque red tint, according to the purity of the liquids and the quantity of copper oxide precipitated.

This is quite sufficient for ordinary purposes. But with liquids containing only a minute quantity of sugar, in a rather dilute form, if we add but very little sulphate of copper, the change of color on boiling may not be sufficiently marked to be satisfactory ; and on the other hand, if we add a quantity of sulphate of copper large enough to make the mixture a decided blue, there is a similar difficulty from another cause. A definite quantity of sugar can only decompose a definite quantity of the

copper salt; and consequently the small amount of copper oxide precipitated may be masked by the blue color of that portion of the liquid remaining undecomposed. It is not easy, therefore, by this means, either to detect sugar in very small amount, or to estimate its absolute quantity in saccharine liquids.

It is claimed by the author of this test * that it will give a visible precipitate on boiling, in a liquid containing one part of grape sugar dissolved in 100,000 parts of water. I have not been able to succeed with it in liquids of so high a degree of dilution, though using every possible care. The most dilute solution in which I have found it yield an appreciable indication is a liquid containing one part of glucose to 10,000 parts of water; and even with this, in order to succeed, we must operate with at least 25 cubic centimetres of the solution—that is, a volume containing $\frac{1}{20}$ of a grain of sugar.

It is easier to detect the presence of sugar in small amount when it is examined in a more concentrated form. According to my experience, the smallest absolute quantity of glucose perceptible by Trommer's test is one cubic centimetre of a watery solution made in the proportion of one part to 2,000, and containing accordingly a little over $\frac{1}{130}$ of a grain of glucose.

Almen's bismuth solution † is far less delicate than the copper test. When freshly prepared the liquid is perfectly clear and colorless. On boiling with a saccharine solution, if glucose be present in decided quantity, it turns to a brownish hue, which on cooling rapidly changes to a nearly pure opaque black, very strongly marked when viewed against a white ground. With one cubic centimetre of a solution containing $\frac{1}{40}$ of a grain of glucose, the blackish color of the mixture after cooling is still

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* Bericht der Königl. Preuss. Akademie der Wissenschaften zu Berlin. 1841, p. 222.

† This solution is made as follows:—

Tartrate of soda and potassa, 160 grains, is dissolved in 4,000 grains of a solution of hydrate of potassa, having the specific gravity 1.33. To this mixture, when warmed, but not boiling, subnitrate of bismuth is added so long as it continues to be dissolved. After cooling, the clear liquor is decanted. The solution must not be made with the chemically pure potassa, which has been prepared with the aid of alcohol, but with the ordinary hydrate of potassa, in sticks.

distinctly perceptible, though it is not opaque; but with $\frac{1}{50}$ of a grain no characteristic reaction takes place.

The hope has sometimes been entertained that the rotation of the polarized ray by saccharine solutions would afford a more delicate test of the presence of sugar than that given by any chemical reaction. This hope, however, has not been realized. In order to produce a decided rotation, the polarized ray must pass through the saccharine solution for a considerable distance, usually about eight inches; and this requires a correspondingly large volume of the liquid under examination.

In Mitscherlich's apparatus, which was intended for medical purposes and is of comparatively simple construction, the tube is 20 centimetres in length, and requires, for application of the test, 28 cubic centimetres of the saccharine liquid. With a solution of cane sugar, made in the proportion of 30 parts to 100, the rotation is 36 degrees. With a solution of cane sugar, one part to 100, it is only from 1 to 2½ degrees. With a solution of glucose (the rotatory power of which is less than that of cane sugar), if made in the proportion of one per cent., the rotation is from zero to 2 degrees. That is to say, with 28 cubic centimetres of such a solution, containing 4⅓ grains of sugar, the glucose is practically inappreciable.

Soleil's saccharimeter is a much more elaborate and delicately constructed instrument. The tube is eight inches in length, and contains 13.75 cubic centimetres of liquid. The standard solution for this apparatus is a liquid containing 26.05 grammes of pure cane sugar dissolved in 100 cubic centimetres of water, —that is, with the sugar in the proportion of 23.68 parts per hundred. With this solution, the scale of the instrument should mark 100 degrees. In order to bring the index to this point, therefore, we must use 13.75 cubic centimetres of the standard solution, containing 55 grains of cane sugar.

Soleil's saccharimeter was intended for the use of sugar manufacturers, and is employed for testing the quantity of good sugar in a tolerably dense solution,—such as will mark somewhere about 30 degrees on the scale of the instrument. In France it is used, as I am informed by Professor Chandler, to test the quality of a raw sugar, by ascertaining its proportion of good sugar available for refining purposes, and in this way to fix its

price for the manufacturer. In this country, at least in some establishments, it is no longer used for that purpose, since the skilled purchaser finds that his inspection of the raw sugar by sight and touch is equally reliable. It is employed mostly for examination of the liquor drained from a crystallizing mass, in order to see how much uncrystallized sugar is still contained in it, and thus to avoid an undue loss in the manufacture.

Notwithstanding the improved construction of this instrument, with saccharine solutions of any grade of strength there is a range of one degree of the scale, in which there is no perceptible difference of color in the polarizing plates. Beside this, on each side of the above range there is a margin of at least one degree, in which the change of color is exceedingly faint, and requires the closest care and attention to be distinguished.

According to my experience, the weakest solution of cane sugar which can be distinguished by the polarizing test with any degree of certainty, is that of one part per thousand; and the necessary volume of such a solution contains $\frac{1}{5}$ of a grain of sugar.

On the other hand, a solution of glucose, made much weaker than one per cent., cannot be recognized with certainty; and a solution of one part per thousand gives no reliable indication whatever of the presence of glucose.

It is evident, therefore, that the polarizing apparatus cannot be relied upon for the detection of glucose in physiological investigations.

By far the most sensitive test for glucose yet discovered is that by Fehling's solution, which is a double tartrate of potash and copper, dissolved in an alkaline liquid, and containing in a given volume a definite quantity of the copper salt.* The extreme sensibility of this solution may be well shown by using it

* Fehling's solution is made as follows:—

Pure crystallized sulphate of copper, 500 grains, is dissolved in about $4\frac{1}{2}$ fluid ounces of water.

Then, neutral tartrate of potassa, 2,000 grains, dissolved in a little water, is mixed with a solution of caustic soda (of the specific gravity 1.12), 8,750 grains.

To this alkaline liquid the copper solution is gradually added, the mixture taking a clear, deep blue color.

The whole is finally diluted with water to the volume of $934\frac{9}{10}$ cubic centimetres, or f. ℥ 31, f. ʒ 5.

in a dilute form. If mingled with 40 times its volume of water and using four cubic centimetres of the mixture, by adding a few drops of a saccharine solution containing $\frac{1}{160}$ of a grain of glucose, and bringing the whole to ebullition, the reaction which follows is brilliant when viewed against a black ground, and quite perceptible both against white and by transmitted light. If a mixture be made of Fehling's solution one part, and water 1,000 parts, by adding $\frac{1}{70}$ of a grain of glucose, there is still a distinct and perfectly characteristic reaction, also visible in all lights, though seen to best advantage against a black ground.

The most effectual way of using this test, for very small quantities of glucose, is to make the following mixture :—

Fehling's solution	1 part.
Water	2 parts.

Of this mixture, five cubic centimetres are placed in a narrow test tube, rather less than half an inch in diameter and about 3½ inches long. The tube should be placed in an oblique position, one inch in front of a background of black glass. It is fixed in this position by means of a cork collar, which embraces it at its upper extremity, and which is held by a metallic ring and screw, attached to a wooden framework behind.

The dilute copper solution is then raised to the boiling-point by the flame of a spirit lamp, care being taken not to apply the flame to the sides of the test tube above the level of the liquid. When the copper solution has thus been brought thoroughly to ebullition, the boiling is allowed to subside; and the saccharine liquid is then immediately added, drop by drop, from another test tube, in which it has already been kept hot for the purpose.

In this way the hot saccharine liquid, flowing down the inclined sides of the test tube, mingles gently with the surface layer of the copper solution ; and when reaction takes place, it is indicated by a thin yellow or orange-colored ring at the surface of the mixture, which contrasts distinctly with the clear blue color of the remainder. Boiling must not be continued after the addition of the saccharine liquid ;—for in that case the minute quantity of copper precipitate, which is perfectly distinct so long as it remains at rest, is broken up and diffused by

the mechanical agitation, and becomes quite imperceptible in the excess of the blue liquid.

The principal condition necessary for success, when testing by this method for sugar in minute quantity, is to have both liquids, at the moment of their admixture, as nearly as possible at the boiling-point without being disturbed by actual ebullition. The apparatus should be illuminated by clear white daylight, coming from a lateral direction. The black background is the best for showing a very delicate reaction; and in this way we can sometimes see distinctly a copper precipitate which would be distinguished with difficulty against a white ground, and quite imperceptible by ordinary transmitted light.

In every case there should be two test tubes, containing equal quantities of the copper solution, placed side by side in a similar position. Both the fluids are to be treated in the same manner, excepting that the saccharine solution is added only to one of them, the other being used simply for comparison, in order to secure accuracy in the results. By this means we avoid the danger of mistake from spontaneous decomposition of the test liquid.*

If Fehling's test be used in the manner now described, with a solution of glucose made in the proportion of one part per 10,000, three drops of the saccharine liquid, added to the hot mixture, will cause the appearance of a faint yellowish ring on the surface of the copper solution; but it is very delicate, and requires for its production every possible care in the manipulations.

With a solution of glucose made in the proportion of one grain to 100 cubic centimetres, one drop,† containing $\frac{1}{1000}$ of

* Fehling's solution is apt to become changed in course of time, if freely exposed to the atmosphere, by the conversion of a portion of its tartaric into carbonic acid; after which it will partially precipitate on boiling, though no sugar be present. To guard against this, it is best to keep the solution in a number of small, well-stoppered bottles, each one of which, except that in actual use, is quite full of the liquid, and is thus protected from the action of the atmosphere. As soon as the solution in use shows signs of alteration, it is thrown away and a fresh bottle substituted. I have found Fehling's solution, when exposed to the air in warm weather, give indications of spontaneous decomposition at the end of a week; while portions of the same liquid, carefully kept in closed and full bottles, remained entirely unchanged for over three months.

† The drops used in these experiments were very nearly equal to one-tenth of a cubic centimetre each.

a grain of glucose, produces, in a few seconds, a slight but distinct reaction. If the same saccharine liquid be diluted with an equal volume of water, one drop, containing $\frac{1}{2000}$ of a grain of glucose, also produces a reaction; but the reaction in this case is rather slow in making its appearance, and is on the extreme limit of certainty. On the other hand, $\frac{1}{3000}$ of a grain of glucose, added in a similar way, causes no recognizable reaction.

That is to say, $\frac{1}{2000}$ of a grain of glucose, *if concentrated in a single drop*, may be detected by Fehling's solution, used in this manner. At the same time, a smaller quantity than $\frac{1}{1000}$ of a grain is hardly available for practical purposes.

It would, however, be very inconvenient to concentrate the fluid extract of an animal tissue to so small a volume as one drop. The better way, on the whole, when we wish simply to determine the presence or absence of glucose in such cases, is to use about one cubic centimetre of the fluid extract, and to add to it one drop of the pure Fehling's solution. This method is sufficiently delicate for all requisite examinations.

If one cubic centimetre of water, containing $\frac{1}{100}$ of a grain of glucose, be placed in a narrow test tube and one drop of Fehling's solution added, the reaction on boiling is prompt and very strong, easily visible in all lights and from a considerable distance.

With a similar quantity of water, containing $\frac{1}{1000}$ of a grain of glucose, and treated as above, the reaction is a little tardy in its appearance, but is perfectly distinct in character, most marked when viewed against a black ground. With weaker solutions the reaction is less distinct, and soon becomes entirely imperceptible.

This accordingly is about the limit of the practical operation of Fehling's test. In delicate examinations the degree of concentration is always of some importance; since the same quantity of glucose, dissolved in double the quantity of water, will often fail to give a reaction, though easily detected in the more concentrated form.

For the purpose of reducing the liver tissue to a state of fine comminution in the shortest possible time, I employed a

machine of simple construction, but very effective in its operation, known as the "crimping machine." It consists of two fluted brass cylinders, placed horizontally side by side, and made to revolve rapidly in opposite directions by means of a crank handle. Each cylinder is six and a half inches in length and one and a half inch in diameter.

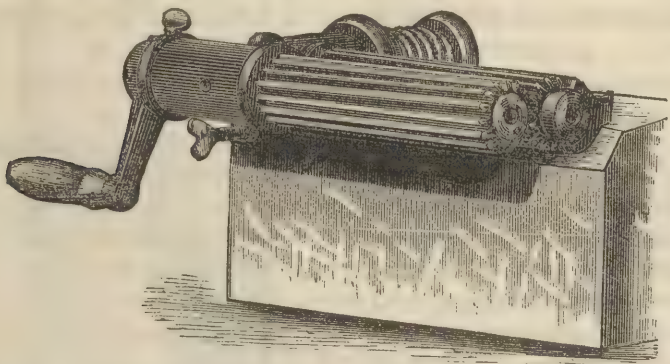

FIG. 1. MACHINE for comminuting the liver tissue; about one-fifth the natural size.

During the revolution of the cylinders, their nearly parallel projections and depressions lock into each other like those of two cog-wheels; their contiguous surfaces, at the point of greatest proximity, being separated by a distance of not more than one-sixteenth of an inch.

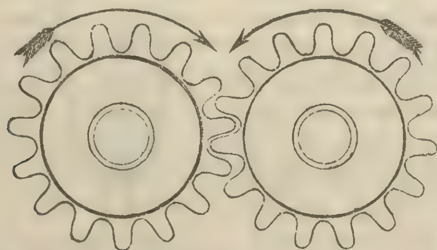

FIG. 2. DIAGRAM showing the cylinders of the comminuting machine in profile; three-quarters the natural size.

When a portion of the liver substance is passed between the rollers of this machine, it is crushed at once into a state of far finer comminution than could be effected by any cutting process

with knife or scissors. The greater part is reduced to the condition of a loose granular débris, and the whole of it is so bruised and lacerated that the contact of alcohol or boiling water will instantly affect its entire mass. By this means from 1,500 to 2,000 grains of the liver tissue may easily be separated from the body of the living animal, thoroughly comminuted, and immersed in alcohol or boiling water, within the space of ten seconds.

The mode of operating which I adopted is as follows: The animal is gently but firmly held upon a table by three assistants, care being taken to prevent any struggling or any undue disturbance of the circulation or respiration. The animal being secured in this position, and quiescent, the abdomen is widely opened by a single stroke of a very sharp knife, the liver seized and drawn downward, a portion of it instantly cut off and passed between the rollers of the comminuting machine into a vessel containing ten fluid ounces of strong alcohol (specific gravity, .820). A fourth assistant meanwhile marks the time consumed in the operation by means of a stop watch, the second hand of which is liberated at the instant the portion of liver is cut off,

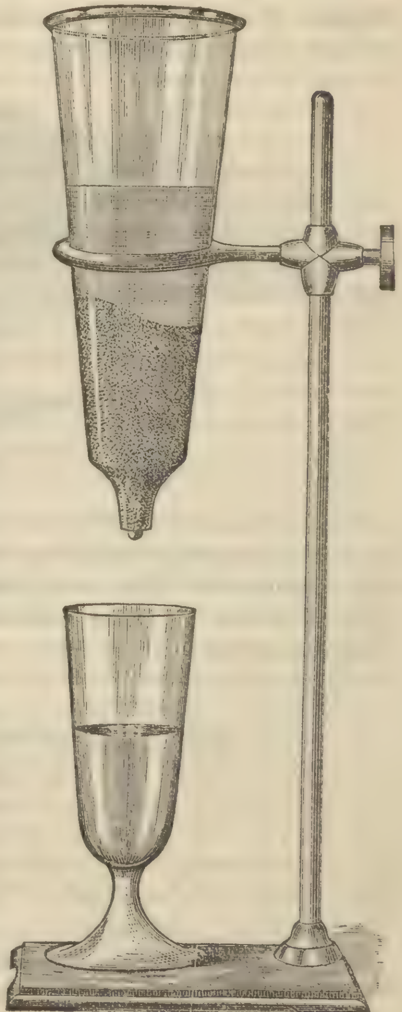

FIG. 3. DISPLACEMENT APPARATUS.

and again stopped when it is comminuted and immersed in the vessel of alcohol.

After remaining for ten minutes in the alcohol, the liver tissue is pounded and ground to a pulp in a porcelain mortar, the pulp thoroughly mixed with the same alcohol, and the mixture then slowly percolated in a displacement apparatus. The moist liver tissue remaining in the apparatus is pressed in a linen bag, and the expressed liquid filtered and added to that which has already passed through by percolation.*

The alcoholic solution is then mixed with an equal volume of water, the turbid mixture clarified in a displacement apparatus with two ounces of coarsely powdered animal charcoal, and evaporated to dryness over the water-bath.† The dry residue is finally dissolved in 50 cubic centimetres of water, again decolorized with one ounce of finely powdered animal charcoal, and filtered. The perfectly clear and colorless watery solution is now examined for sugar, by placing one cubic centimetre of the liquid in a narrow test tube, against a black ground, adding one drop of Fehling's solution, and raising the mixture to the boiling point.

In order to determine the proportion of sugar present in the liver tissue, one cubic centimetre of Fehling's solution, diluted with water to five times its volume, is placed in a large-sized test tube against a white ground, and raised to the boiling-point. While the ebullition continues, a measured quantity of the liver extract is slowly added, drop by drop, until all the copper ox-

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* In some of the first experiments, more alcohol was added in the percolating apparatus, until twenty ounces in all had passed through; but this was subsequently found to be unnecessary, since ten ounces will extract all the glucose contained in the quantity of liver tissue usually employed, or, at least, sufficient to give a well-marked sugar reaction. The addition of a larger quantity of alcohol is even detrimental in one respect, as it dissolves out more fatty matter, and thus renders the subsequent clarification of the liquid more difficult.

† A more direct method would be to evaporate the alcoholic solution to dryness, and immediately dissolve the residue in water. But if treated in this way the final watery solution is very turbid, and its clarification with animal charcoal is a process of extreme difficulty. The operation is greatly facilitated by proceeding as indicated above. The best way is to mix the alcoholic solution directly with the animal charcoal, and afterward to add the water. With careful management, the liquid will then pass through perfectly clear.

ide present has been precipitated, and the remaining liquid, when filtered, has no longer any blue color. The composition of Fehling's solution is such that, to accomplish this result with one cubic centimetre of the test liquid, it requires exactly .077 of a grain of glucose; which quantity was accordingly present in the portion of liver extract employed. From this is calculated the amount of glucose in the whole fifty cubic centimetres of liver extract, representing a certain quantity of liver tissue; and thence the proportion of sugar, per thousand parts, in the liver tissue itself. The quantity of liver substance, used in each experiment, was ascertained by comparing the volume of the comminuted liver tissue and alcohol with that of another, previously weighed, portion of liver, treated in the same way and mixed with the same quantity of alcohol.

A second method is to employ boiling water, instead of alcohol, for the purpose of preventing post-mortem fermentation. The portion of liver cut out, in the manner already described, is passed through the comminuting machine directly into a vessel containing thirty fluid ounces of water in active ebullition. With this quantity of water, the temperature of the mixture, on immersing the bruised liver, does not fall at any time below 210° Fahrenheit, and actual ebullition recommences immediately. The boiling is continued for five minutes, after which the liver tissue is ground in a mortar to a fine pulp, and again mixed with the same water. The mixture is next boiled down to about two fluid ounces, mixed with five times its volume of alcohol and filtered; after which the alcoholic solution is treated as in the former process.

In each case it is essential that the final watery solution be absolutely clear and colorless. Otherwise, in testing for small quantities of glucose, it is sometimes difficult to be certain whether a genuine reduction have taken place or not: and especially in using the volumetric method for quantitative determination, the extraneous matters present interfere with the test, and prevent our fixing the precise point at which all the copper of the test liquid has been reduced.

I have now experimented in this manner upon twenty dogs. In four of the cases, the method employed was that by boiling water; in the remaining sixteen cases, that by alcohol. The

animals were examined four, eight, twelve, and twenty-four hours after feeding; the food consisting always of the fresh or cooked meat of the bullock's heart. The longest time which elapsed, from the separation of the liver to its immersion in the alcohol or boiling water, was 13 seconds; the shortest time was 3 seconds. The average time was 6¼ seconds. In every instance the final watery solution gave a decided and perfectly unmistakable sugar reaction; amply sufficient, in all cases in which it was attempted, to allow the quantitative determination of the glucose by the volumetric method. The proportion of glucose in 1,000 parts of the liver tissue was thus ascertained in one-half the cases. In the remainder its presence only was determined, without regard to actual quantity.

The following is a list of the experiments, with their results:

Experiment.	Time after Feeding.	Time consumed in taking out Liver.	Process of Treatment.	Proportion of Glucose in 1,000 parts of Liver.
No. 1.	4 hours.	13 seconds.	Alcohol.	Glucose.
No. 2.	4 hours.	7 seconds.	"	"
No. 3.	4 hours.	10 seconds.	"	"
No. 4.	4 hours.	4 seconds.	"	"
No. 5.	8 hours.	7 seconds.	"	"
No. 6.	8 hours.	6 seconds.	"	"
No. 7.	4 hours.	5 seconds.	Boiling water.	"
No. 8.	12 hours.	6 seconds.	" "	"
No. 9.	12 hours.	8 seconds.	" "	"
No. 10.	12 hours.	5 seconds.	" "	"
No. 11.	4 hours.	9 seconds.	Alcohol.	2.093
No. 12.	4 hours.	5 seconds.	"	0.804
No. 13.	8 hours.	7 seconds.	"	1.750
No. 14.	8 hours.	3 seconds.	"	1.510
No. 15.	12 hours.	5 seconds.	"	1.810
No. 16.	12 hours.	5 seconds.	"	4.175
No. 17.	12 hours.	7 seconds.	"	1.830
No. 18.	12 hours.	3 seconds.	"	4.375
No. 19.	24 hours.	5 seconds.	"	3.850
No. 20.	24 hours.	4 seconds.	"	2.675

In no single instance, in these experiments, was Fehling's test employed for the detection of sugar, without a similar quantity of the test liquid being boiled at the same time, to make sure that it was not liable to spontaneous precipitation. The volu-

metric method was applied with every care, the liver extract being added very slowly, and the fluid, after precipitation, being nearly always filtered twice, in order to determine the exact point at which its decolorization was complete.

There is no doubt that the quantity of glucose in the liver increases immediately after death; although, according to my experiments, this increase is not always so rapid as might be inferred from the general statements of some writers. In the following cases, its quantity, per thousand parts, was determined at various periods after death:

Proportion of Glucose in the Liver.

	At the end of	*Per* 1,000 *parts.*
Exp. No. 15....	5 seconds	1.810
	15 minutes	6.792
	1 hour	10.260
Exp. No. 19....	5 seconds	3.850
	6 hours	11.458
Exp. No. 20....	4 seconds	2.675
	1 hour	11.888
	4 hours	13.361
	12 hours	15.351

It might perhaps be doubted whether the glucose thus existing in the liver at the moment of death may not be due to the arterial blood with which the organ is supplied, rather than an ingredient belonging to the hepatic tissue. This, however, is not the case. The question may be settled by examining, at the same time with the liver or immediately afterward, some other abdominal organ equally well supplied with arterial blood. The spleen was selected for this purpose; and in three cases was taken out within ten minutes after the excision of the liver, and treated in precisely the same manner by the alcohol process, with the following result:

Proportion of Glucose per 1,000 *parts.*

	At the end of	*In the*	
Exp. No. 14....	3 seconds........	Liver....	1.510
	10 minutes.......	Spleen...	0
Exp. No. 19....	5 seconds........	Liver....	3.850
	10 minutes.......	Spleen...	0
Exp. No. 20....	4 seconds........	Liver....	2.675
	10 minutes.......	Spleen...	0

In the last-mentioned experiments, the watery extract of the spleen, treated in the same way with that of the liver, gave no reduction whatever on boiling with Fehling's solution; but in each case, after being concentrated over the water-bath to one-tenth of its volume, it yielded an uncertain sugar reaction, entirely too small for quantitative determination.

From these results I think we may legitimately draw the following conclusions:

I. Sugar exists in the liver at the earliest period at which it is possible to examine the organ after its separation from the body of the living animal.

II. The average quantity of sugar existing in the liver at this time is at least two and a half parts per thousand.

III. The liver sugar thus found does not belong to the arterial blood with which the organ is supplied, but is a normal ingredient of the hepatic tissue.

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